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穗花杉属的核形态及其系统位置的探讨

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Karyomorphology and relationships of *Amentotaxus* Pilg.

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Abstract In this paper, two species in *Amentotaxus* Pilg. from the mainland of China, *A. argotaenia* and *A. yunnanensis*, were karyomorphologically studied. They commonly showed the interphase nuclei of the complex chromocenter type and the mitotic prophase chromosomes of the interstitial type. Their karyotypes were formulated as K(2n) = 36 = 8m + 28T, both belonging to Stebbins' 1B type and with the N. F. value (number of fundamental) being 44. The chromosome number and karyotype of *A. yunnanensis* were reported here for the first time. Karyomorphological features of *Amentotaxus*, especially the N. F. value, together with other evidence, have indicated its close relationship with *Torreya* within the Taxaceae.

Key words *Amentotaxus* Pilg.; Karyomorphology; Systematic position

Amentotaxus Pilg. is a small genus of five species and one variety (Ferguson, 1989, 1985; Lan, 1984; Cheng, Fu, 1978). Three species and one variety of this genus have been recorded in China. *A. formosana* Li is endemic to Taiwan, *A. yunnanensis* Li occurs in SE Yunnan, SW Guangxi, SW Guizhou of China and northern Vietnam, and *A. argotaenia* (Hance) Pilg. is widely distributed from SE Xizang (Tibet), along the southern part of the Mt. Qingling, to southeast China, and its variety, *A. argotaenia* var. *brevifolia* K. M. Lan, has been only recorded from Guizhou.

The systematic position of *Amentotaxus* has long been in dispute (Xi, 1986; Hu *et al.*, 1986; Chen, Wang, 1984; Hu, 1983; Keng, 1969; Li, 1952; Florin, 1951, 1948). *A. argotaenia* (Hance) Pilg. was first described by Hance under *Podocarpus* of the Podocarpaceae. Pilger (1903) transferred this species to the genus *Cephalotaxus* of the Cephalotaxaceae based on its complex male inflorescence, but in 1916, he established a monotypic genus for it, i.e. *Amentotaxus* Pilg., based on its unique long staminate inflorescence (Pilger, 1916). In his last systematic treatment of this genus, Pilger (1926) still recognized *Amentotaxus* as a distinct genus of the Cephalotaxaceae. It was Kudo and Yumamoto (1931) who first raised *Amentotaxus* to a familial status. However, this treatment was not adopted by Florin (1951, 1948), who positioned this genus in the Taxacecae. Since then, the accumulating data from all lines of research on *Amentotaxus* (Hu *et al.*, 1986; Xi, 1986; Chen, Wang, 1984) have become flourishing, but its systematic position still remains unsolved (Page, 1990).

Previous reports of chromosome numbers of *Amentotaxus* have long been controversial. Sugihara (1943) reported the chromosome number of *A. argotaenia* as n = 11, a number also found in

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Torreya. However, Chuang and Hu (1963) reported the chromosome number of *A. formosana* as 2n = 14 under the name *A. argotaenia*, a number different from those of *Taxus*, *Pseudotaxus* and *Torreya* of the Taxaceae, and also that of *Cephalotaxus*. As a result, they supported the establishment of the Amentotaxaceae. Guan *et al*. (1993) reported the chromosome number of *A. argotaenia* as 2n = 40, a number close to that of some species of *Podocarpus*, and thus they considered that *Amentotaxus* should be placed in the Podocarpaceae. It is obvious that authentic chromosome data may be of critical significance for the consideration of the systematic position of *Amentotaxus*.

In this paper, two species of *Amentotaxus*, i.e. *A. argotaenia* and *A. yunnanensis*, were karyomorphologically studied in order to confirm the chromosome number of *Amentotaxus* and have a better understanding of its systematic position.

1 Materials and Methods

A. argotaenia was collected from Changsha City (alt. 800 m), Hunan Province and *A. yunnanensis* from Malipo County (alt. 1100 m), Yunnan Province. The voucher specimens, Zhou 9691201 for the former species and Zhou 9581202 for the latter, were deposited in the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences (KUN).

For the observation of chromosomes, roots of female individuals of both species were pretreated with 0.1% colchicine for 16 hr, and then fixed for 30 min in Carnoy's fluid (absolute alcohol: glacial acetic acid = 3:1). After being macerated in a mixture of 1 mol/L hydrochloric acid and 45% glacial acetic acid (1:1) at 60℃ for two min, they were stained and squashed with 1% aceto-orcein.

Karyomorphological classification of interphase nuclei and mitotic prophase chromosomes followed Tanaka (1977, 1971). The symbols for the description of karyotypes followed Li and Chen (1985). Karyotype classification followed Stebbins (1971).

2 Results and Discussion

The two species studied were very similar in karyomorphology of interphase nuclei and of mitotic prophase chromosomes. In the interphase nuclei (Fig. 1: A), one large and many small darkly stained chromocenters were observed. According to Tanaka (1977, 1971), the interphase nuclei were categorized to be complex chromocenter type. In the mitotic prophase chromosomes (Fig. 1: B), hetero- and eu-chromatic segments were distinguishable, and the darkly stained heterochromatic dots were found at the distal chromosome ends. Therefore, the prophase chromosomes belonged to the interstitial type (Tanaka, 1977).

Metaphase chromosomes of *A. argotaenia* were counted to be 2n = 36 (Fig. 1: C), and the karyotype was formulated as 2n = 8m + 28T (Fig. 1: E), belonging to Stebbins' 1B type. The ratio of the longest chromosome to the shortest one was 2.71 and the N.F. value was 44. Four secondary constrictions were obviously found in the distal regions of the 6th and the 28th chromosome pairs. Metaphase chromosomes of *A. yunnanensis* were also counted to be 2n = 36 (Fig. 1: D), and the karyotype was also formulated as 2n = 8m + 28T (Fig. 1: F), belonging to Stebbins' 1B type. The N.F. value was also 44, but the ratio of the longest chromosome to the shortest one decreased to 2.59. Secondary constrictions were found in the short arms of the 6th, the 9th, and the 12th chromosome pairs. Karyotypic data of both species are given in Table 1.

The chromosome number of *A. argotaenia*, 2n = 36, as reported here, was different from the previous count n = 11 reported by Sugihara (1943), and the count 2n = 40 by Guan *et al*. (1993).

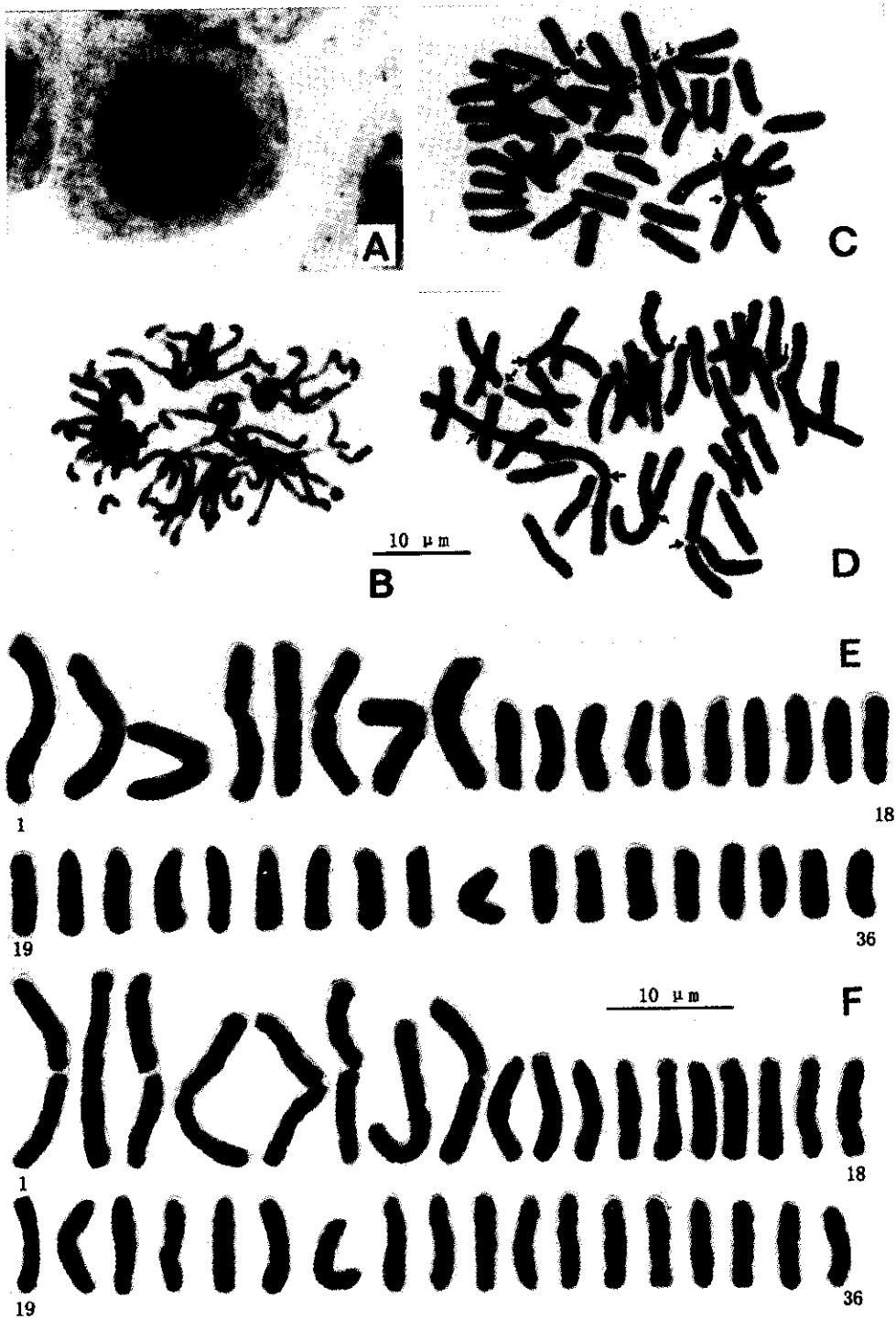

Fig. 1 A: Interphase nuclei of *Amentotaxus argotaenia*. B: Mitotic prophase chromosomes of *A. argotaenia*. C: Metaphase chromosomes of *A. argotaenia*. D: Metaphase chromosomes of *A. yunnanensis*. E: Karyotype of *A. argotaenia*. F: Karyotype of *A. yunnanensis*. (Arrows in C and D indicate the m-chromosomes)

Although the chromosome number of this species reported by us is different from that by Guang *et al*., the N.F. value was both 44. In the procedure of chromosome preparation of *A. argotaenia*, we found that four m-chromosomes of this species could be easily broken from the centromeric sites and then formed eight "T-like" chromosomes. This is very possibly the reason why Guan *et al*. (1993) miscounted the chromosome number of *A. argotaenia* as 2n = 40 but obtained the correct N.F value of 44. We agree with Guan *et al*. (1993) that the material used by Chuang and Hu (1963) for chromosome observation under the name *A. argotaenia* should be actually identified as *A. formosana*. As the chromosome number in the same genus in gymnosperms are usually very constant, we guess that Chuang and Hu's report of the chromosome number of 2n = 14 for *A. formosana* was most likely wrong. The chromosome number of *A. formosana* needs to be reexamined.

Table 1　Karyotypic data of two species of *Amentotaxus*

A. argotaenia K(2n) = 36 = 8m + 28T							*A. yunnanensis* K(2n) = 36 = 8m + 28T						
No.	RL	AR	PC	No.	RL	PC	No.	RL	AR	PC	No.	RL	PC
1	5.01	1.26	m	19	2.40	T	1	4.98	1.08	m	19	2.32	T
2	4.81	1.10	m	20	2.37	T	2	4.72	1.04	m	20	2.29	T
3	4.61	1.06	m	21	2.35	T	3	4.72	1.04	m	21	2.29	T
4	4.54	1.24	m	22	2.34	T	4	4.72	1.04	m	22	2.29	T
5	4.37	1.15	m	23	2.34	T	5	4.50	1.14	m	23	2.25	T
6	4.20	1.26	m	24	2.30	T	6	4.43	1.04	m	24	2.25	T
7	4.03	1.17	m	25	2.29	T	7	4.33	1.16	m	25	2.25	T
8	4.00	1.19	m	26	2.29	T	8	4.10	1.01	m	26	2.22	T
9	2.57		T	27	2.29	T	9	2.69		T	27	2.21	T
10	2.56		T	28	2.29	T	10	2.51		T	28	2.21	T
11	2.54		T	29	2.17	T	11	2.45		T	29	2.21	T
12	2.54		T	30	2.08	T	12	2.40		T	30	2.18	T
13	2.52		T	31	2.07	T	13	2.37		T	31	2.16	T
14	2.51		T	32	2.05	T	14	2.37		T	32	2.15	T
15	2.47		T	33	2.03	T	15	2.35		T	33	2.11	T
16	2.47		T	34	1.95	T	16	2.34		T	34	2.00	T
17	2.47		T	35	1.88	T	17	2.34		T	35	1.98	T
18	2.46		T	36	1.85	T	18	2.32		T	36	1.92	T

RL: relative length; AR: arm ratio; PC: position of centromere

As aforementioned, *Amentotaxus* was once placed in different families, including the Taxaceae (Fu *et al*., 1999; Cheng, Fu, 1978), Cephalotaxaceae (Page, 1990) and Podocarpaceae (Guan *et al*., 1993), or treated as a distinct family of its own (Xi, 1986; Kudo, Yamamoto, 1931). Gross-morphologically, *Amentotaxus* is more or less related to the Taxacecae based on their common real cone with fleshy aril, although some characters, such as the opposite and decussate leaves, the complex compound male spike, the female organs at the top of long pedicels, and the cupular aril with the seed tip out, seem not to support the inclusion of *Amentotaxus* in the Taxaceae. Nevertheless, Ye *et al*. (1996) found that *Amentotaxus* has epigeal seedlings, elongated hypocotyle with the lower part thickened, and fleshy cotyledons in the development of seedling. The seedling morphology of *Amentotaxus* implied its close relationship with *Torreyra* . Furthermore, ana-

tomical data of *Amentotaxus* also indicated its close relationship with *Torreyra* (Hu, 1983). Embryological features of *Amentotaxus*, however, suggested its close affinity with *Austrotaxus* of the Taxaceae (Chen, Wang, 1984; Wang *et al*., 1979). Pollen morphology of *Amentotaxus* is different from that of its presumed relatives and supports its independent familial status (Xi, 1986). Although Page (1990) had placed *Amentotaxus* in the Cephaloaxaceae, he felt that it might be more reasonable to treat the genus as an independent family.

Both *Taxus* and *Pseudotaxus* of the Taxaceae were found to have the karyotype of 2n = 24 = 22m + 2T, with the N.F. value being 46 (Gu *et al*., 1998a; Chen, 1996, 1990). The genus *Cephlotaxus* of the Cephalotaxaceae has 2n = 24 = 22m + 2sm(sat), with the N.F. value being 48 (Gu *et al*., 1998a, 1998b). Although the chromosome number of *Podocarpus* of the Podocarpaceae has variation to a certain degree, most species of this genus have 2n = 38 (Hizume *et al*., 1988). The combination of chromosome characters of *Amentotaxus*, i.e. the chromosome number of 2n = 36, the karyotype consisting of 8m and 28T and the N. F. value of 44, shows that this genus is cytologically readily distinguishable from the Cephalotaxaceae and Podocarpaceae. In the chromosome evolution of gymnosperms, the Robertson translocation seems to be very common, which can often result in the change of the chromosome number, but does not cause the change of the N.F. value. Although the genus *Torreya* has a different chromosome number of 2n = 22 from 2n = 36 of *Amentotaxus*, their N.F. value is both 44. Therefore, the same N.F. value indicates that *Amentotaxus* might have a close relationship with *Torreya*. The difference in their chromosome number might have resulted from the Robertson translocation. *Amentotaxus* has 8 m- and 28 T-chromosomes. *Torreya* has 22 m-chromosomes. The T-chromosomes of *Amentotaxus* might be produced from m-chromosomes through Robertson translocation.

Based on cytological evidence, therefore, we consider that *Amentotaxus* should be a member of the Taxaceae and might have close relationship with *Torreya*.

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摘要　研究了穗花杉 *Amentotaxus argotaenia* 和云南穗花杉 *A. yunnanensis* 的核形态。它们的有丝分裂间期核都为复杂染色中心型，前期染色体为中间型，中期染色体数目均为 2n = 36，核型相似，均为 K(2n) = 36 = 8m + 28T，核型不对称性属于 1B 型，N.F.值为 44。云南穗花杉的核型为首次报道。结合其它学科的资料，认为穗花杉属同榧属 *Torreya* 比较接近。

关键词　穗花杉属；核形态；系统位置

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